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Characteristics of the amino acids as components of a peptide hormone sequence

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In the living organism the polypeptide chains of proteins are used for the most diverse purposes: structural support and protection, catalysis of a wide range of chemical reactions, energy transduction, food storage, transport, and—among many others—regulation and co-ordination by information transfer (including humoral transmission by hormones). Molecules with the varied properties required for these multifarious functions are built up of a mere 20 units: the protein-constituent amino acids, connected primarily in a single structural mode (a linear sequence) but finally arranged in space in intricate ways to molecules of the required size, shape and properties. Although the peptide hormones fulfil such a highly specialised role, there is nothing to distinguish, *a priori*, their sequences from other polypeptide or protein sequences with different biological functions, or no function at all.

THE AMINO ACIDS

The 'proteinogenic' amino acids, though few in number, exhibit between them a remarkable range of chemical, physical and steric features. They are arranged in figure 1.1 in such a way that lines can be drawn to indicate their classification according to various properties. For instance, the sidechains may be hydrophilic (outside the hook-shaped line) or hydrophobic (within the hook); glycine, lying on the line, is taken as the reference amino acid for this purpose.

Again, some sidechains are chemically inert (those to the left of the broken line), while those to the right show varying kinds and degrees of chemical reactivity and may be capable of substitution, hydrogen bond or salt formation, oxidation, etc. Other classifications are shown by the use of frames. Some of the reactive sidechains are neutral, but others (shown by the lower right-hand frame) are charged either positively or negatively in the physio-

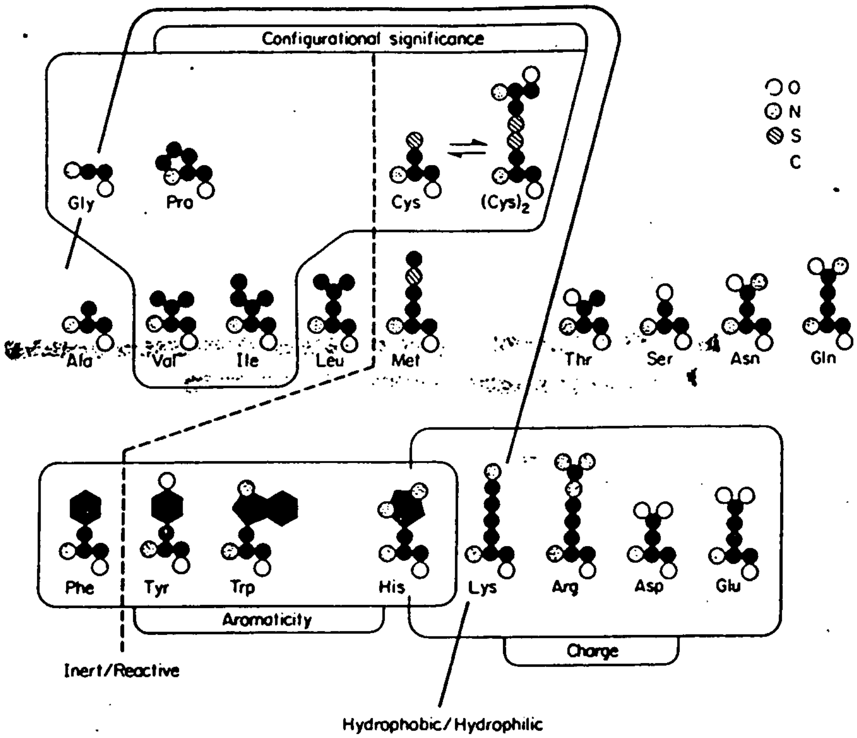

FIGURE 1.1 Schematic representation of the 20 amino acyl residues found in proteins, and their classification by certain properties; for details see text

logical pH range. Histidine may or may not be protonated under physiological conditions. The two basic amino acids (lysine and arginine) differ in their equilibrium constants (arginine being the stronger base) and so do the two acidic amino acids (aspartic being a somewhat stronger acid than glutamic). Thus, between them, the charged amino acids encompass a wide range of pK values.

The lower left-hand frame encloses those sidechains which contain aromatic structures and exhibit corresponding special properties (π-electron interactions). Finally, the frame at the top draws attention to amino acids with special steric properties which affect the way in which the peptide chain can be arranged in three dimensions (its conformation or secondary structure). Glycine, with no obtruding sidechain, offers a particularly high degree of conformational freedom, whereas the bulky, β-branched sidechains of valine and isoleucine severely restrict the way in which the peptide chain may fold. A still greater degree of constraint is imposed by the rigid cyclic structure of proline. Proline has also the special property that the peptide bond in which its imino group participates may have either the *cis* or the *trans* conformation, whereas all other peptide bonds are normally

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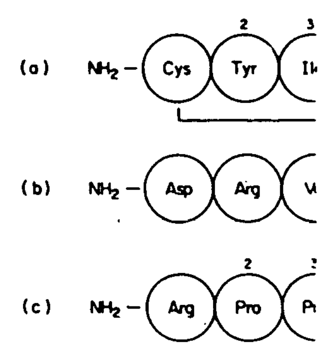

FIGURE 1.2 Sequences of

confined to the *trans* geometry. The formation of disulphide bridges between cysteine sidechains provides an even more positive way of covalently cross-linking and thereby stabilising the three-dimensional arrangement of peptide chains.

It will be noted that most amino acids occur in two or more of the classifying 'boxes'—in other words, each generally has several different structural features which may be utilised in protein building alternatively or simultaneously. As a result, it is impossible to attach a unique significance to any residue in a sequence. A given amino acid will not by any means have the same 'significance' in different peptide sequences, or even in different positions of the same sequence.

For instance, isoleucine is found both in position 3 of oxytocin (figure 1.2a) and in position 5 of angiotensin II (figure 1.2b). Whereas in angiotensin it may be replaced, without appreciable loss of biological activity, by other β-branched amino acids provided they are equally lipophilic, in oxytocin the steric requirements are much more stringent and even replacement by the diastereomeric alloisoleucine causes a drastic fall in activity (Rudinger, 1972; Jorgensen and Weinkam, 1973).

Another illustration that sidechain 'significance' depends on 'context' is provided by the molecule of bradykinin (figure 1. 2c). Replacement of the proline in position 3 by alanine does not affect the potency of the peptide as assayed on the rabbit blood pressure; obviously, the special steric properties of proline are of no significance in this position. On the other hand, the same substitution of the proline in position 2 reduces the activity to 0.5 per cent, and in position 7 to 0.1 per cent (Schröder and Hempel, 1964). Moreover, if sarcosine (*N*-methylglycine) rather than alanine is used to replace proline at these sites, the resulting analogues are considerably more active than the alanine derivatives (they retain, respectively, 50 and 30 per cent of the activity of the parent compound; Yanaihara *et al.*, 1966).

We may therefore conclude that in positions 2 and 7 of the bradykinin sequence it is the *N*-alkylation of the nitrogen in the peptide backbone

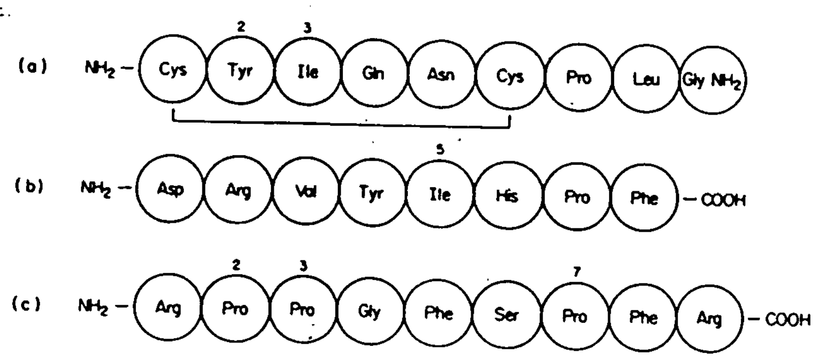

FIGURE 1.2 Sequences of oxytocin (a), angiotensin II (b) and bradykinin (c)

which is the important feature of the proline structure rather than, for example, the presence of its ring or its lipophilic properties.

SEQUENCE AND CONFORMATION

The humoral mechanism of information transfer requires that the effect of a hormone on its receptor be both sensitive and specific. These requirements can be met only by hormone–receptor binding based on multiple interactions of complementary sites: it is a *pattern* on the hormone molecule which is 'recognised' by the receptor in the binding process. Generally, parts of several amino acids of the sequence will participate in forming this pattern. In peptide molecules which are short or conformationally very flexible, or both, the pattern will often involve the sidechains of amino acids which are close together in the primary peptide sequence ('continuate' or 'synchnologic' read-out (Schwyzer, 1972), analogous to 'sequential' determinants in antigens (Sela, 1969)).

However, in peptides whose conformation is stabilised either by a sufficient number of intramolecular, non-covalent sidechain interactions or by disulphide bonds, or both, the critical topochemical pattern may be made up of groups widely separated in the linear sequence (Hofmann and Katsoyannis, 1963; Schwyzer, 1963; Rudinger and Jošt, 1964) ('discontinuate' or 'rhegnylogic' read-out, analogous to 'conformational' determinants).

Whereas in the first case the often-cited analogy to a 'message' written in linear, alphabetic script is valid, a topochemical arrangement of the second type is better likened to Chinese writing: it is the *pattern* of the character which conveys the meaning (figure 1.3) and not the (prescribed) order in which the brush strokes are made.

In either case it should be noted that the conformation of the hormone molecule in its interaction with the receptor need not be identical with its conformation in solution (cf. Rudinger and Jošt, 1964; Rudinger, 1972),

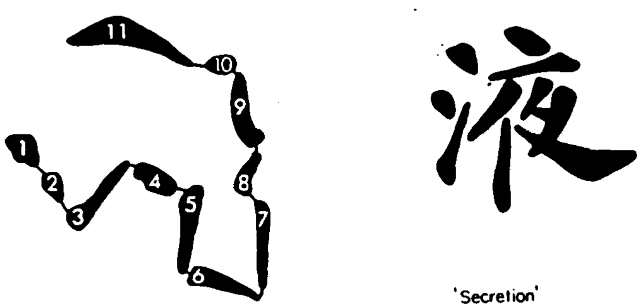

FIGURE 1.3 Eleven brush-strokes in the order in which they are made but in random pattern (left), and in the pattern in which they make up the Chinese character for 'secretion'

although major, energetically disfavou admittedly unlikely.

SIGNIFIC

In a given molecule some amino acid: 'significance' to their inclusion in the in recognition by, and binding to, the ı existence of this pattern is dependen intramolecular interactions, as discuss acids or sequences contributing to this less 'significant' for the biological ac generally, sequences contributing to tl affect its transport and distribution, m non-receptor sites, etc., may signific: In defining the relation between sequer into account all these contributions, aı in receptor binding*.

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although major, energetically disfavoured structural rearrangements are admittedly unlikely.

SIGNIFICANCE

In a given molecule some amino acids or sequences obviously owe their 'significance' to their inclusion in the pattern which is directly involved in recognition by, and binding to, the receptor. However, the fact that the existence of this pattern is dependent on a conformation stabilised by intramolecular interactions, as discussed above, implies that other amino acids or sequences contributing to this conformational stability will be no less 'significant' for the biological activity of the molecule. Even more generally, sequences contributing to those properties of the peptide which affect its transport and distribution, metabolic transformations, binding to non-receptor sites, etc., may significantly modify its biological activity. In defining the relation between sequence and activity it is necessary to take into account all these contributions, and not merely those directly involved in receptor binding*.

Two separate events may be conceptually distinguished in hormone–receptor interaction: binding (recognition) and stimulation (initiation of the signal which eventually leads to the observed response). In some of the models proposed to account for hormone action, the process of stimulus generation is actually identical with the process of binding. These are variants of the 'allosteric' model (Monod, Changeux and Jacob, 1963), which have in common the assumption that binding of hormone at one site modifies the activity of a second, topologically distinct site on the same molecule, probably by inducing a conformational change. In other models (the 'participation' type; Rudinger, Pliška and Krejčí, 1972) binding and stimulus generation are two distinct molecular events.

The properties of a series of oxytocin analogues modified at the tyrosine residue (position 2) illustrate a possible experimental approach to this problem and favour a 'participation' model for oxytocin. As shown in figure 1.4, replacement of the hydroxyl group of this tyrosine by various substituents leads, in a graded manner, to loss of oxytocin-like activity and to the appearance of inhibitor properties. Yet the binding affinity, determined from pharmacological parameters (pD_2 and pA_2), is practically the same for the whole series of analogues, suggesting that the region of the molecule around position 2 is involved in stimulus generation but contributes little to binding (see Rudinger *et al.*, 1972). Moreover, we have recently found that substitution *ortho* to the tyrosine hydroxyl group—for example, by iodine or methyl—also gives rise to inhibitors: evidently the hydroxyl group is

*Obviously, these various functions need not and, in general, will not be attributable to separate and distinct sequences, but particular amino acids, sequences or topochemical regions may participate in several of them.

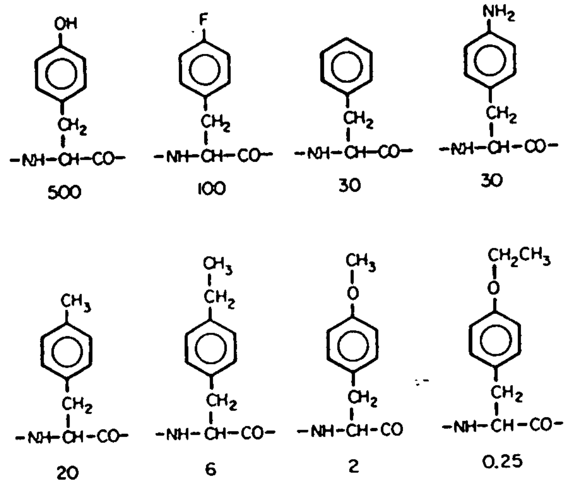

FIGURE 1.4 Replacement of the tyrosine sidechain (top left) by other, modified sidechains in analogues of oxytocin. The figures give the uterotonic activity in IU/mg under standard assay conditions (see Rudinger *et al.*, 1972; Marbach and Rudinger, 1974a)

displaced from its functionally required alignment by the substituents, but once more the binding affinity, as measured by the inhibitory constants, remains high (Marbach and Rudinger, 1974b).

CONCLUSIONS

The significance of particular amino acids and sequences for different aspects of biological activity cannot be predicted *a priori* but must be determined from case to case by painstaking experimental study. The careful design of synthetic analogues, and their evaluation in biological systems which permit separate analysis of the various phases of hormone action, is still the best way of obtaining such information.

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